STRUCTURAL STUDIES ON STEM BROMELAIN ISOLATION, CHARACTERIZATION AND ALIGNMENT OF THE CYANOGEN BROMIDE FRAGMENTS

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1. Introduction

Stem bromelain [EC 3.4.22.4.], a proteolytic enzyme of pineapple stem, has a mol. wt. of about 28 000 [1]. In this paper, we describe the isolation and the alignment of three peptides obtained by means of cyanogen bromide cleavage. The tryptic peptides from the parent protein and its CNBr fragments were also isolated by a combination of column and paper separation techniques. The peptides have been characterized by amino acid analyses and end group determinations. Isolation of overlapping peptides to determine the alignment of all of the tryptic peptides is still in progress. However, the partial sequence we report here covers unique portions of the molecule, such as the amino and carboxyl-termini, the branching point for the carbohydrate moiety, and the catalytic center residues, so that it deserves comparison with the sequence of the corresponding portions of papain [2].

2. Materials and methods

Stem bromelain was prepared by the method of Takahashi et al. [1]. Purification of CNBr fragments: Enzyme protein was dissolved in 70% formic acid to make a concentration of 1 g/10 ml. 2 g of CNBr was added and the mixture was incubated overnight at 37°C. The reaction mixture was diluted 10-fold with water and lyophilized. Maleylation of the CNBr-treated protein with 2.4 g of maleic anhydride was carried out by the method of Butler and Hartley [3]. The material was desalted by further chromatography on Bio-Gel P-4 and lyophilized. Sulfitolysis of the above protein was subsequently done according to the procedure of Iwanaga et al. [4]. The sulfitolyzed protein separated from the reagents by means of gel filtration was fractionated on a column of Sephadex G-50 in 0.05 M ammonium bicarbonate buffer, pH 8.0. Four abundant components, named I, II, III and IV were further purified by rechromatography under the same conditions. Preparation and analysis of tryptic peptides: Stem bromelain (1 g) was dissolved in 36 ml of 5 M guanidine hydrochloride at pH 8.0, and 20 mg of iodoacetic acid was added to make a molar ratio of reactive sulfhydryl group to the reagent of 1 : 3, and the reaction mixture was maintained at pH 8.0 overnight at 25°C in the dark. Reduction and carboxymethylation of thus obtained SH-blocked bromelain was carried out by the method of Crestfield et al. [5] with slight modification. The product, R-Cm-bromelain, was allowed to react with maleic anhydride, and then digested with TPCK-treated trypsin for 2 h at an enzyme/substrate ratio of 1% (w/w). The CNBr fragments were digested by trypsin in a similar way. Tryptic peptides thus obtained were separated and further purified using column chromatography on Bio-Gel P-4, Sephadex G-50 and anion exchanger DE-52, and/or high-voltage paper electrophoresis and descending paper chromatography. Amino terminal sequence of each peptide was determined by the Edman's method [6]. Amino acid compositions of peptides were analyzed by an amino acid analyzer [7].

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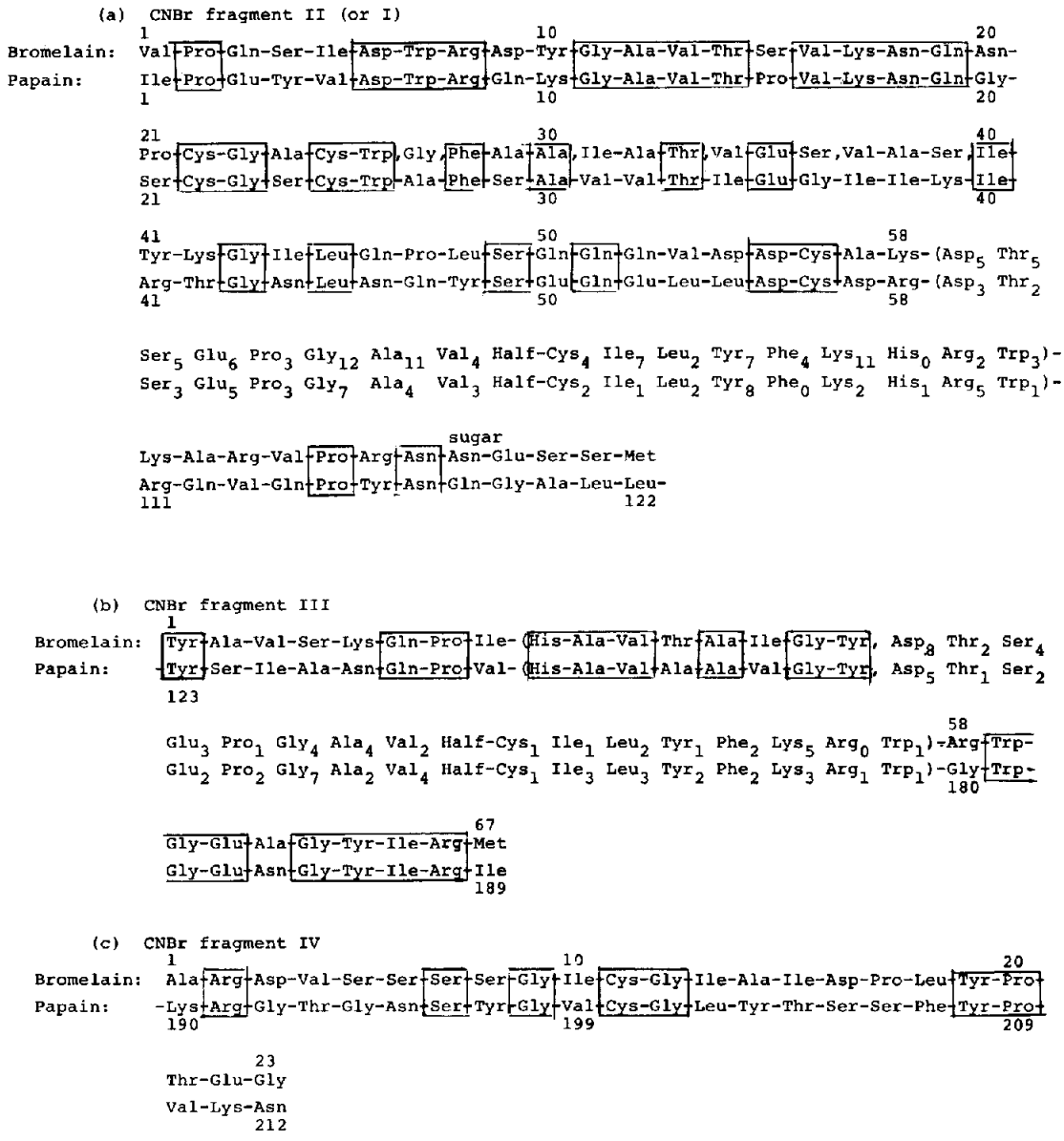

Fig.1. Summary of amino acid sequence studies on CNBr-cleaved fragments. The sequence for papain was taken from Drenth et al. [2]. The residue numbers for papain refer to the positions from the amino end group of the parent protein, while those for stem bromelain show the positions on each CNBr fragment. Homologous amino acid residues are outlined. The following residue and sequences of stem bromelain have been reported: in fragment II Val^{1} – Gln^{3} [1], Ser^{15} – Trp^{26} [1], Ala–Arg–Val–Pro–Arg–Asn–Asn (sugar) –Glu–Ser–Ser–Met [9]; in fragment III His–Ala–Val–Thr–Ala–Ile–Gly–Tyr [10]; and the carboxyl-terminal Gly [1].

3. Results and discussion

After sulfitolysis of the CNBr-treated stem bromelain, four major fragments I-IV could be isolated. On molecular weight determination using a column of Sephadex G-75 [8], fragment I emerged at the void volume of the column. Its amino acid composition appeared to be identical with that of fragment II. Fragment I, therefore, represents an aggregated product of fragment II. The mol. wt. of fragment II was determined to be 17 000 by polyacrylamide gel electrophoresis in the presence of sodium dodecylsulfate. The amino acid compositions of fragments II, III and IV gathered together are generally in good agreement with the reported composition of the parent protein [1]. Although the sulfitolysis gave three different CNBr fragments, the CNBr cleavage without sulfitolysis gave only two fractions, corresponding to fragment II (or I) and III plus IV. The result shows that three of the four disulfide bridges in native bromelain were involved in fragment II and the other linked fragment III with fragment IV. Fragment II is located at the N-terminus and fragment IV at the C-terminus of the protein. The latter contains no homoserine residue. The location of fragment III in-between is deduced from the amino acid sequences of two overlapping, methionine-containing tryptic peptides: Asn–Asn (sugar)–Glu–Ser–Ser–Met–Tyr–Ala–Val–Ser–Lys and Met–Ala–Arg. The partially established amino acid sequence in stem bromelain is shown in fig.1 (a), (b) and (c) in comparison with that of papain [2]. The homology extends not only around the reactive sulfhydryl group [1,10], but also all over the molecule (about 40%). However, being a glycoprotein, stem bromelain may be expected to have a portion or portions of the molecule, most probably in the vicinity of the carbohydrate group, whose amino acid sequence is less homologous with any part of the molecule of papain which is a simple protein.

References

[1] Takahashi, N., Yasuda, Y., Goto, K., Miyake, T. and Murachi, T. (1973) J. Biochem. 74, 355.
[2] Drenth, J., Jansonius, J. N., Koekoek, R. and Wolthers, B. G. (1971) in: The Enzymes, Vol. 3, (P. D. Boyer, ed.) p. 485. Academic Press, New York and London.
[3] Butler, P. J. G. and Hartley, B. S. (1972) in: Methods in Enzymology, Vol. 25, (C. H. W. Hirs and S. N. Timasheff eds.) p. 191. Academic Press, New York and London.
[4] Iwanaga, S., Henschen, A. and Blombäck, B. (1966) Acta Chem. Scand. 20, 1183.
[5] Crestfield, A. W., Moore, S. and Stein, W. H. (1963) J. Biol. Chem. 238, 662.
[6] Iwanaga, S., Wallén, P., Gröndahl, N. J., Henschen, A. and Blombäck, B. (1969) Eur. J. Biochem. 8, 185.
[7] Spackman, D. H., Moore, S. and Stein, W. H. (1955) Anal. Chem. 30, 1190.
[8] Whitaker, J. R. (1963) Anal. Chem. 35, 1950.
[9] Kito, K. and Murachi, T. (1969) J. Chromatog. 44, 205.
[10] Husain, S. S. and Lowe, G. (1970) Biochem. J. 117, 341.